

Please replace the paragraph beginning at page 5, line 23, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The present invention also provides a method for treating apoptotic cell formation in a subject. The method includes introducing to a subject exposed to or at risk of exposure to an agent that damages DNA a composition that includes an effective amount of a polypeptide having pyrimidine glycosylase activity, preferably pyrimidine glycosylase/AP lyase activity, and includes a targeting sequence, preferably, an exogenous targeting sequence.

Please replace the paragraph beginning at page 9, line 5, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

As used herein, "damaged base" and "damaged bases" refers to structural deviations in nucleoside-5'-monophosphates present in a eukaryotic cell's genomic DNA. One type of structural deviation is a covalent joining of the adjacent pyrimidines through the formation of a cyclobutane ring structure at the C5 and C6 positions. Another type of structural deviation is an imidazole ring fragmentation of a purine (either adenine or guanine). The location of such structural deviations in a cell's genomic DNA is referred to as a "lesion." As used herein, "genomic DNA" refers to the DNA present in the nucleus and the mitochondria of a cell. Damaged bases preferably arise from , for instance, UV radiation, ionizing radiation, oxidative stress, alkylation damage, and deamination. Examples of lesions include *cis-syn* and *trans-syn II* cyclobutane pyrimidine dimers, FapyA and FapyG (Lloyd, *Mutat. Res.*, 408, 159-170 ((1998), and Lloyd, *Progress in Nucleic Acid Research and Molecular Biology*, 62, 155-175 (1999)).

Please replace the paragraph beginning at page 10, line 1, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Whether a polypeptide has pyrimidine glycosylase/AP lyase activity can be determined by measuring the ability of the polypeptide to incise a target polynucleotide containing damaged bases in the presence of a buffer. The target polynucleotide contains damaged bases, preferably, UV radiation induced pyrimidine dimers. An example of a target polynucleotide is disclosed in the Examples. Preferably, the target polynucleotide is present at a concentration of from about 0.1 nM to about 10 nM. The putative glycosylase/AP lyase is present at a concentration of from about 0.01 nM to about 100 nM. Buffers in which a glycosylase/AP lyase is active are suitable for the assay. Preferably, the buffer includes about 25 mM  $\text{NaH}_2\text{PO}_4$ . Preferably, the pH is from about 6.5 to about 7.5, more preferably about 6.8. Preferably the buffer contains from about 10 mM NaCl to about 125 mM NaCl, more preferably about 100 mM NaCl. Preferably the buffer contains from about 1 mM EDTA to about 10 mM EDTA, more preferably about 1 mM EDTA. Preferably the buffer contains from about 0.01 mg/mL bovine serum albumin (BSA) to about 1 mg/mL BSA, more preferably about 0.1 mg/mL BSA. Preferably, the temperature of the assay is about 37°C. The assay can be carried out for at least about 10 seconds to no greater than about 8 hours. Preferably, the assay is about 30 minutes. A polypeptide having pyrimidine glycosylase/AP lyase activity will cause the mobility of the target polynucleotide to change relative to the polynucleotide that has not been exposed to the polypeptide. The polypeptide may be present in a crude cellular extract, preferably isolated, more preferably, purified. Since polypeptides identified in this assay as having pyrimidine glycosylase/AP lyase activity function on UV-irradiated DNAs, these polypeptides identify cyclobutane pyrimidine dimers, and are likely to be active on other UV-induced photoproducts including FapyA and Fapy G.

Please replace the paragraph beginning at page 11, line 15, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The present invention further includes polypeptides having pyrimidine glycosylase activity, preferably pyrimidine glycosylase/AP lyase activity, and amino acid identity with the amino acid sequence of SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43,

preferably SEQ ID NO:41 or SEQ ID NO:42. Amino acid identity is defined in the context of a comparison between a polypeptide and SEQ ID NO:41 or SEQ ID NO:42, and is determined by aligning the residues of the two amino acid sequences (i.e., a candidate amino acid sequence and the amino acid sequence of SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43) to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. A candidate amino acid sequence is the amino acid sequence being compared to an amino acid sequence present in SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43. A candidate amino acid sequence can be isolated from a microbe or a microbe harboring a virus, or can be produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, two amino acid sequences (i.e., the candidate amino acid sequence and the amino acid sequence present in SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43) are compared using the Blastp program of the BLAST 2 search algorithm, as described by Tatusova, et al. (*FEMS Microbiol Lett* 1999, 174:247-250), and available at [www.ncbi.nlm.nih.gov/gorf/bl2.html](http://www.ncbi.nlm.nih.gov/gorf/bl2.html). Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x\_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, amino acid identity is referred to as "identities." Preferably, a polypeptide having pyrimidine glycosylase activity has an amino acid sequence having, in increasing order of preference, at least about 15 % amino acid identity, at least about 30 % amino acid identity, at least about 40 % amino acid identity, at least about 50 % amino acid identity, and most preferably, at least about 60 % amino acid identity to SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43.

Please replace the paragraph beginning at page 15, line 28, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Optionally, a polypeptide of the present invention further includes a series of consecutive amino acids encoding a domain that facilitates the isolation, preferably purification, of the polypeptide. An "isolated" polypeptide or polynucleotide means a polypeptide or polynucleotide that has been either removed from its natural environment, produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, a polypeptide or polynucleotide of this invention is purified, i.e., essentially free from any other polypeptide or polynucleotide and associated cellular products or other impurities. For instance, domains that are useful in the isolation of a polypeptide that has glycosylase activity, preferably glycosylase/AP lyase activity, include a histidine domain (which can be isolated using nickel-chelating resins), an S-peptide domain (which can be isolated using an S-protein, see Kim, J.-S. et al. *Protein Sci* 1993 2:348-356), and a chitin binding domain (which can bind to chitin beads, see Chong et al. *Gene*, 192, 271-281 (1997) and Watanabe et al. *J. Bacteriol.*, 176, 4465-4472 (1994)). Preferably, the domain is present at the carboxy terminal end of the polypeptide. Preferably, the domain can be cleaved from the remainder of the polypeptide (e.g., the polypeptide having pyrimidine glycosylase activity, preferably pyrimidine glycosylase/AP lyase activity, fused to a targeting sequence, preferably an exogenous targeting sequence) by the use of a protease or self-cleaving sequence.

Please replace the paragraph beginning at page 24, line 13, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

A preferred cosmetic formulation is a sunscreen composition. A sunscreen can advantageously additionally include at least one further UVA filter and/or at least one further UVB filter and/or at least one inorganic pigment, preferably an inorganic micropigment. The UVB filters can be oil-soluble or water-soluble. Advantageous oil-soluble UVB filter substances are, for example: 3-benzylidenecamphor derivatives, preferably 3-(4-methylbenzylidene)camphor and 3-benzylidenecamphor; 4-aminobenzoic acid derivatives, preferably 2-ethylhexyl 4-(dimethylamino)benzoate and amyl 4-(dimethylamino)benzoate;

esters of cinnamic acid, preferably 2-ethylhexyl 4-methoxycinnamate and isopentyl 4-methoxycinnamate; derivatives of benzophenone, preferably 2-hydroxy-4-methoxybenzophenone, 2-hydroxy-4-methoxy-4'-methylbenzophenone and 2,2'-dihydroxy-4-methoxybenzophenone; esters of benzalmalonic acid, preferably di(2-ethylhexyl) 4-methoxybenzalmalonate. Advantageous water-soluble UVB filter substances are, for example: salts of 2-phenylbenzimidazole-5-sulphonic acid, such as its sodium, potassium or its triethanolammonium salt, and the sulphonic acid itself; sulphonic acid derivatives of benzophenones, preferably 2-hydroxy-4-methoxybenzophenone-5-sulphonic acid and salts thereof; sulphonic acid derivatives of 3-benzylidenecamphor, such as, for example, 4-(2-oxo-3-bornylidenemethyl) benzenesulphonic acid, 2-methyl-5-(2-oxo-3-bornylidenemethyl) benzenesulphonic acid and salts thereof. The list of further UVB filters mentioned which can be used in combination with the active agent(s) according to the invention is not of course intended to be limiting.

Please replace the paragraph beginning at page 27, line 10, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Treatment of the conditions described herein can be prophylactic or, alternatively, can be initiated after the development of a condition described herein. Treatment that is prophylactic, for instance, initiated before a subject manifests symptoms of a condition described herein and/or before exposure to an agent that damages DNA, for instance, UV light, oxidative stress, alkylation damage and deamination, preferably UV light, is referred to herein as treatment of a subject that is "at risk" of developing the condition. Accordingly, administration of a composition can be performed before, during, or after the occurrence of the conditions described herein. Treatment initiated after the development of a condition may result in decreasing the severity of the symptoms of one of the conditions, or completely removing the symptoms. Non-limiting examples of subjects particularly suited to receiving the composition are those who may be exposed to natural or artificial UV irradiation, individuals having genetic deficiencies in polypeptides involved in DNA repair (for instance, those suffering from xeroderma

pigmentosum), and individuals who are immunosuppressed due to disease states (such as AIDS) or transplantation.

Please replace the paragraph beginning at page 28, line 15, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Whether the repair rate of damaged bases in a cell is increased can be determined by, for instance, assaying for the amount of damaged DNA in cells using a variety of techniques including coding sequence-specific repair assays (Bohr et al., *Cell*, 40, 359-369 (1985)), and photoproduct removal as determined by ELISA assays using antibodies directed against *cis-syn* dimers (Clarkson et al., *Mutation Res.*, 112, 287-299 (1983)). Alternatively, when human cells are used, the removal of lesions can be assayed by quantitative PCR assay that is specific for human mitochondrial DNA (see Ballinger et al., *Exp. Eye Res.*, 68, 765-772 (1999), and Ballinger et al., *Circ. Res.*, 86, 960-966 (2000)). For instance, ex vivo cells can be exposed to an agent that damages DNA, preferably UV light, and treated with a composition including a polypeptide of the present invention. After a period of time sufficient to allow repair, the amount of damaged DNA in the cells can be determined and compared to the same type of cell that was not treated with the polypeptide. The presence of less damaged DNA in the cell treated with the polypeptide relative to the cell not treated indicates the polypeptide increases the repair rate of DNA. The repair rate of damaged DNA in *in vivo* cells may also be determined. For instance, an animal can be exposed to an agent that damages DNA, and treated with a composition including a polypeptide of the present invention. After a period of time sufficient to allow repair, skin biopsies are prepared and the amount of damaged DNA determined and compared to skin biopsies obtained from animals not treated with the polypeptide. The presence of less damaged DNA in cells in the biopsies treated with the polypeptide relative to cells in the biopsies not treated indicates the polypeptide increases the repair rate of DNA. Commonly accepted *in vivo* models are available for testing whether a polypeptide will increase the repair rate of DNA (for human models, see, for instance, Yarosh et al., *Photochem. Photobiol.*, 69,

136-140 (1999); for animal models, see, for instance, Mitchell et al., *J. Invest. Dermatol.*, 95, 55-59 (1990)).

Please replace the paragraph beginning at page 29, line 12, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The present invention further provides methods for treating mutagenesis in a cell, preferably a skin cell, in response to an agent that damages DNA, preferably UV light. In this aspect of the invention, mutagenesis rates are decreased. Mutagenesis results when repair of damaged DNA does not occur and, upon replication of the DNA, a different base is inserted. The method includes introducing to a skin cell exposed to or at risk of exposure to an agent that damages DNA a composition that includes an effective amount of a pyrimidine glycosylase, preferably a pyrimidine glycosylase/AP lyase, that includes a targeting sequence. Whether the rate of mutagenesis in a cell is reduced can be determined by, for instance, *hprt* mutagenesis assays (O'Neill et al, *Mutat. Res.*, 45, 103-109 (1977)). Briefly, the measurement of mutagenesis using an *hprt* assay involves the selection of mammalian cells that are resistant to the killing effects of 6-thioguanine through a mutation in the *hprt* coding sequence. The assay relies on an inability of *hprt*- cells to activate 6-thioguanine for incorporation into DNA that results in cell killing. All cells with wild type *hprt* are killed upon 6-thioguanine selection. The cells can be *in vivo* or *ex vivo*. The rate of mutagenesis in cells treated with a polypeptide of the present invention can be determined and compared to the rate of mutagenesis in cells not treated. The presence of a lower mutagenesis rate in treated cells relative to untreated cells indicates the polypeptide decreases the mutagenesis rate of DNA.

Please replace the paragraph beginning at page 30, line 21, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The present invention is also directed to methods for treating tumor formation in a cell, preferably a skin cell, in response to an agent that damages DNA, preferably UV light. In

this aspect of the invention, tumor formation is decreased. The types of tumors that may occur in response to an agent that damages DNA include actinic keratosis, basal cell carcinoma, squamous cell carcinoma, and melanoma. The method includes introducing to a skin cell that is at risk of developing a tumor in response to an agent that damages DNA a composition that includes an effective amount of a pyrimidine glycosylase, preferably a pyrimidine glycosylase/AP lyase, that includes a targeting sequence. Cells at risk of developing a tumor in response to an agent that damages DNA include cells exposed to or at risk of exposure to an agent that damages DNA. Whether the formation of tumors in an animal is reduced can be determined by the use of animal models, for instance mice, that have been exposed to solar simulated light or exposure to sunlight. Solar simulated light is light having a spectral profile which is similar to natural solar irradiation, i.e. the emission spectrum of a solar simulator looks similar to spectrum of a solar noon day. Wavelengths of light include ~295-400 nm so is inclusive of UVA, UVB but not UVC which does not get through the ozone (see, for instance, Yoon et al., *J. Mol. Biol.*, 299, 681-693 (2000)). The presence of a tumor can be determined by methods known in the art, and typically include cytological and morphological evaluation. The cells can be *in vivo* or *ex vivo*, including obtained from a biopsy. The rate of tumor formation in cells treated with a polypeptide of the present invention can be determined and compared to the rate of mutagenesis in cells not treated. The presence of a lower rates of tumor formation in treated cells relative to untreated cells indicates the polypeptide decreases tumor formation.

Please replace the paragraph beginning at page 31, line 16, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Another aspect of the present invention is directed to treating the formation of apoptotic cells, preferably apoptotic skin cells, in response to an agent that damages DNA, preferably UV light. Apoptotic cells are cells undergoing, or that have undergone, programmed cell death. In this aspect of the invention, the formation of apoptotic cells is decreased. The method includes introducing to a skin cell exposed to or at risk of exposure to an agent that damages DNA a composition that includes an effective amount of a pyrimidine glycosylase,



preferably a pyrimidine glycosylase/AP lyase, that includes a targeting sequence. Whether the formation of apoptotic cells is reduced can be determined by, for instance, assays that detect apoptotic cells. Such assays include immunohistochemistry using antibodies against apoptotic-specific polypeptides associated with apoptotic cells, including, for instance, anti-caspase 8, anti-procaspase 9, and anti-G3PDH antibodies. Such antibodies are known to the art, and are available from, for instance, Trevigan (Gaithersburg, MD) and Sigma (St. Louis, MO). The cells can be *in vivo* or *ex vivo*, including obtained from a biopsy. The formation of apoptotic cells in cells treated with a polypeptide of the present invention can be determined and compared to the formation of apoptotic cells in cells not treated. The presence of a lower apoptosis rate in treated cells relative to untreated cells indicates the polypeptide decreases the formation of apoptotic cells.

Please replace the paragraph beginning at page 42, line 20, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The expression and purification of the recombinant proteins from transformed ER2566 *E. coli* was performed as suggested by the manufacturer (New England BioLabs). Briefly, cultures of 0.7 O.D.<sub>600</sub> were induced with isopropyl-1-thio- $\beta$ -D-galactoside (IPTG) (final concentration 0.3 mM) for seven hours at 20-25°C. The cells were harvested by centrifugation at 5000 x g for 10 min and the cell pellet resuspended in buffer A (20 mM HEPES, pH 8.0, 0.5 M NaCl, 0.1 mM EDTA, 0.1% Triton X-100). The cells were disrupted using a French Press with constant pressure of 9000 p.s.i. and the cell lysate cleared of cellular debris by centrifugation at 12,000 x g for 30 min. The cleared lysate was applied to a chitin bead column (New England BioLabs) that had been pre-equilibrated with buffer B (20 mM HEPES, pH 8.0, 0.5 M NaCl, 0.1 mM EDTA). The column was washed with buffer B and flushed with buffer C (20 mM HEPES, pH 8.0, 50 mM NaCl, 0.1 mM EDTA) containing 30 mM DTT. Following overnight incubation at room temperature, the recombinant protein was eluted with buffer B and the collected fractions were monitored for the target protein by polyacrylamide-SDS gels and staining with Coomassie Brilliant Blue R-250. The fractions that contained the recombinant

protein were pooled, dialysed in buffer E (25 mM sodium phosphate, pH 8.0, 50 mM NaCl, 0.1 mM EDTA), and concentrated using an Amicon YM10 membrane (Millipore, Bedford, MA). The purity and the size of the recombinant proteins were assessed using 15% polyacrylamide-SDS gels along with purified wild type-T4-pdg and wild type-cv-pdg as controls. The gel was run at 15 mA for 5 hours in 1x Tris-Glycine buffer and subsequently stained with Coomassie Blue R-250. The addition of the ML18 corresponded to the mobility shift seen in MLS18-PDGs when compared to the wild-type PDGs. All purified recombinant proteins were stored in dialysis buffer E at 4°C.

Please replace the paragraph beginning at page 46, line 2, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Mitochondria from non-transfected cells (control) and transfected cells expressing MLS18-cv-PDG and MLS18-T4-PDG were purified as previously described (Yang et al., *Science* 275, 1129-1132 (1997)). Briefly,  $\sim 1.1 \times 10^7$  cells were trypsinized, collected by centrifugation at 750 x g for 5 minutes, and washed once with ice cold PBS. The cell pellet was then resuspended in sucrose-buffer A (20 mM HEPES, pH 7.5, 10 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.1 mM PMSF) and 250 mM sucrose. The cells were lysed with a homogenizer so that at least 70% of cells were broken. The homogenate was centrifuged twice at 750 x g at 4°C for 10 minutes to remove unbroken cells, nuclei, and cell debris. The supernatant from this step was centrifuged at 7,800 x g at 4°C for 30 minutes and the mitochondrial pellet washed twice with sucrose-buffer A. For preparation of mitochondria lysate for DNA nicking assays, the mitochondrial pellet was lysed in for 5 minutes at room temperature 0.2 ml buffer A (without sucrose) containing 0.5% CHAPS. The sample was then centrifuged at 15,000 x g for 30 minutes at 4°C to obtain a clear lysate. Small amounts of this clear lysate and its dilutions were tested for DNA nicking activity. No pyrimidine dimer specific activity was detected in the control HeLa S3 cells, while two independent clones, one expressing MLS18-T4-pdg and one expressing MLS18-cv-pdg, both showed dimer-specific nicking. These

**Preliminary Amendment**

**Page 12 of 13**

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For: DNA REPAIR POLYPEPTIDES AND METHODS OF USE

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data prove that the MLS sequences target these enzymes to the mitochondria and that they were processed to yield active enzymes.

Please replace the paragraph beginning at page 46, line 26, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

II. Nuclear Targeting of cv-pdg and T4 pdg